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FOURTH REPORT

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TROPICAL RESEARCH LABORATORIES

AT THE

GORDON MEMORIAL COLLEGE

KHARTOUM

VOLUME A.—Medical

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Herpetomonas lygæi

Considered from a Protozoological and Medical Aspect

BY

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The relationship existing between the genera Trypanosoma, Herpetomonas, Crithidia and various diseases which affect man, is still somewhat in a state of chaos. Some of the parasites of these genera are known to have as their hosts various insects and biting flies, but the rôle played by these as transmitting agents of such diseases as Kala-azar, Trypanosomiasis, and Oriental Sore still requires further elucidation. While I was engaged in studying a certain type of Herpetomonad which was found to infest the alimentary tract of the dura plant bug in the Sudan, a paper was published by Patton[1] describing a similar type of flagellate which is parasitic in the *Lygæus militaris* found in India. The same insect, known as the dura plant bug, is also ubiquitous in the Sudan, and a rich material enabled one to study in detail the morphology and life-cycle of the parasite infesting its intestinal tract. *Lygæus militaris* is a brilliantly coloured hemipteron* which exists as a pest in various parts of the Sudan, doing considerable damage to the dura or millet crops of the country. Its ravages, however, are not confined to dura alone, for it has been found feeding on various plants, notably the *Calotropis procera*. By means of a piercing apparatus it punctures the plant and destroys its vitality by withdrawing the sap.

Characters of *Lygæus militaris*

If the alimentary tract of this bug be dissected out, it will be seen to consist of a very short œsophagus opening into a large sacculated crop in which peristaltic movements can be observed taking place. The crop opens into a narrow and long mid-intestine which ends in a bulbous dilatation. At the other end of this dilatation a short ileum extends posteriorly and connects with a dilated colon in which are found the openings of the Malpighian tubes. From the colon, the remainder of the alimentary tract is represented by a short tube opening into the rectum.

Material and Methods Employed

In order to study the different stages of the flagellate in the various portions of the alimentary tract of *Lygæus militaris*, the method employed, and one which gave satisfactory results, was to dissect out the alimentary tract of the bug in normal saline solution and then to transfer the various portions, with their contents intact, to several watch-glasses containing fresh normal salt solution, where emulsions were made and transferred to slides, spread, and then stained in a suitable manner. Fresh hanging-drop preparations were also made in order to study the various stages of the flagellate.

A wet method of fixation was usually employed before the films were stained, Leishman's modification of Romanowsky or iron hæmatoxylin being the stains used. The latter was chiefly used to differentiate the nuclear and chromatic structures present in the herpetomonad.

Fixation of films

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[1] Patton, W. S. (1908), *Herpetomonas lygæi. Arch. f. Protistenk.*

* *Vide* Second Report of these Laboratories, fig. 56, *p.* 95.

Patton in his paper described the life-cycle of this herpetomonas as occurring in three different stages which he names the preflagellate, flagellate and post-flagellate. All these stages can be respectively seen in various parts of the alimentary tract of *Lygæus militaris*.

If a hanging-drop preparation of the crop contents be examined, adult flagellates can be observed, but not in very great numbers. They appear to be more plentiful in the small mid-intestine.

Activity of the flagellates

They are actively motile and display various types of movement, the commonest of which is a jerky, wriggling motion, with the flagella end forward, the latter vibrating vigorously; this movement is often followed by a peculiar gliding one in which the flagellum is in a state of quiescence, standing out from the body in a straight line. At other times the flagellum displays lashing movements, and occasionally the herpetomonad moves forward by means of its posterior end, while the flagellum trails behind. When the latter becomes attached to any object the body appears to bend, and sways to and fro in a rhythmical manner, at the same time twisting on itself. The twist may occur both ways from right to left and vice versa. Sometimes the flagellate appears to rotate on its own axis showing a peculiar action resembling the movements of a "Catherine wheel." All these movements are brought about by the presence of myonemes in the body of the flagellate. Waves of contraction pass along these body myonemes from the posterior to the anterior end. These waves of contraction can be well seen in the herpetomonad just prior to its encystment, when its movements become very sluggish.

As each wave of contraction passes along the body, small refractile granules can be seen alternately advancing and receding down the flagellum for about a third of its length.

The Preflagellate Stage

Plate XI., figs. 1–6

If the crop contents be examined for the presence of cysts, very few will be found; this is due to the fact that such a rapid development of the cyst occurs in the crop that practically only flagellates are found. The early cystic stage is really best studied in the fæces where they are more numerous.

The preflagellate stage in Leishman-stained specimens are small, oval, pyriform or round cells, $2-4\mu \times 1-1{\cdot}5\mu$, with a pale blue cytoplasm, limited by a sharply defined edge or periplast. The pink trophonucleus is most often situated at the rounded end of the cell, and is either circular or bean-shaped, measuring about 1μ. When stained by iron hæmatoxylin, and suitably differentiated, a distinct nuclear membrane is apparent, but at this stage of the parasite chromosomes are rarely seen.

The kinetonucleus occupies a position anterior to the trophonucleus, and appears as a dark staining rod or dot in the more pointed or anterior portion of the cell. Vacuoles are occasionally to be found in the cell cytoplasm, and at this stage the flagellum may appear arising from the vicinity of the kinetonucleus and reaching the exterior as a pink streak.

Development—first stage

The first stage in the further development of the parasite is a general enlargement both in length and breadth, and the cell cytoplasm appears very markedly reticulated. The trophonucleus enlarges in size, and may occupy half, or more than half, of the cell area. The parasite, instead of appearing as a short compact little cell, now resembles a delicate structure more or less pear-shaped and measuring about 6μ in length. A clear area now begins to form round the kinetonucleus, which at this stage begins to elongate,

and shows a slight constriction in its middle, producing a dumb-bell like appearance. Division of the kinetonucleus then occurs, followed by division of the trophonucleus, and longitudinal division of the cell wall and its cytoplasm, and two separate parasites, each containing a tropho- and a kinetonucleus, are thus formed. The anterior ends of the parasite are usually directed towards one another or lie in close apposition. Each parasite may again go through the same process of division, and four or more parasites be thus formed. The further stage of development continues in the crop. Most of these parasites pass into the stage of flagellation while others may yet divide again before becoming flagellates. In those parasites that undergo further development, the anterior end becomes elongated and the kinetonucleus moves farther from the trophonucleus. This is followed by elongation of the posterior end of the parasite and the development of a vacuole anterior to the kinetonucleus; from this vacuole a pale pink filament can be seen developing. This represents the flagellum (Plate XI., figs. 7–9). Longitudinal division may then occur, the line of cleavage usually commencing at the anterior end of the parasite. Many of the parasites at this stage show marked vacuolation, especially at their posterior extremity, and appear as short stumpy tadpole forms with thickened kinetonuclei and flagella. The trophonucleus is often stretched out with its long axis running transverse to the parasite. All these various stages of development up to mature flagellates may be seen in the crop; the mid-intestine, however, shows the largest number of typical adult flagellates. These adult flagellates measure on an average 26μ in length and 2μ in breadth. In appearance they are somewhat cigar-shaped, with sharp pointed posterior ends, and blunt rounded anterior ends from which the flagella extrude. The cytoplasm is vesicular in structure, and often contains pink-coloured granules, especially in the posterior portion of the parasite (Plate XI., figs. 10–14).

Further stages

If an iron hæmatoxylin stain be employed it is quite apparent that these granules are not of a chromatin nature, for the cytoplasm is stained very uniformly in this area, showing, perhaps, a slight reticular structure, but rarely anything that could be considered chromatic granules. It is difficult to say what these granules represent; in all probability they are of the nature of reserve foodstuff. Porter[1] is inclined to think they represent metabolic changes as they are usually present when the parasite is in a highly nutritive medium, but, when deprived of this medium, the granules present are but few in number. This view is supported by the fact that herpetomonads present in the gut of bugs that have been starved for a few days show practically no granules in their cytoplasm, while those herpetomonads removed from bugs that have been recently feeding have a marked granular cytoplasm. The trophonucleus is, as a rule, circular in shape and occupies more or less a central position in the parasite. Around its margin is a definite nuclear membrane containing chromosomes; these are more apparent in specimens stained by Giemsa than by Leishman's stain. In iron hæmatoxylin specimens the nuclear membrane is well brought out but no central karyosome is evident.

A nuclear membrane

The kinetonucleus occupies a position anterior to the trophonucleus, and is usually 4μ distant from it. In adult flagellates it is invariably rod-shaped, running transverse to the long axis of the parasite, and is placed about 4μ from the anterior extremity. The cytoplasm between the tropho- and kinetonuclei stains quite uniformly and shows no evidence of the spiral "Doppelfaden" described by Prowàzek in *H. muscæ domesticæ*.

Immediately in front of the kinetonucleus is a small achromatic space, on the anterior margin of which there appears a slight thickening of the cell cytoplasm in the form of a small rod or dot. This is rarely seen except in films fixed by a wet method.

[1] Porter, A. (1909), "The life cycle of *Herpetomonas jaculum*," Leger. *Parasitology*, Vol. II., pp. 367-391.

Origin of Flagellum

The flagellum arises from this small dot or basal granule which, according to Minchin,[1] ought to be termed the blepharoplast. After its origin from the blepharoplast it occupies a median longitudinal position in the parasite and passes out of its anterior extremity as a fine tapering filamentous structure, stained a deep red colour. It varies in length, and in adult flagellates it is about twice as long as the body of the parasite.

When the flagellate reaches the adult stage, longitudinal division usually occurs and two flagellates result. Prior to division, enlargement of the tropho- and kinetonuclei takes place, the latter becoming thickened and a constriction appearing in its middle. A second small flagellum, arising close to the original one, then makes its appearance, and one is inclined to think that its origin is a separate one and that it is not due to a splitting of the original flagellum. Many observations on hanging-drop preparations were made, but it was impossible to come to any definite conclusion concerning this much debated point. With the appearance of the second flagellum the kinetonucleus divides transversely into two separate portions, and this is followed by a similar division of the trophonucleus. A line of cleavage first forms between the sites of origin of the two flagella and extends in a posterior direction along the body of the parasite; when completed, two parasites result. These again may undergo a second division, passing through more or less identical processes. The parasite, therefore, multiplies by longitudinal division. Many observations were carried out with hanging-drop preparations for the purpose of ascertaining whether conjugation ever took place, but in no instance was this ever detected. In stained preparations one was often inclined to think that parasites of different sexes were present. Frequently, large stout forms measuring 30 μ in length and 3 μ in breadth and often devoid of a flagellum were present; they were granular in structure and in Leishman-stained preparations it was sometimes difficult to make out any differentiation between trophonucleus and kinetonucleus. They stood out in marked contrast to the more attenuated typical forms of flagellates.

In view of the fact, however, that conjugation was never observed, one must look on these stout forms as degenerated parasites.

Post-Flagellate Stage

Plate XI., fig. 16

This is characterised in the first place by the herpetomonads becoming attached to the lining epithelium of the colon, and if this portion of the gut be teased out on a slide and stained, large masses of flagellates will be seen fixed to the gut wall by means of their flagella, their posterior ends lying free. They appear to have undergone no change in structure from the typical flagellates found in the mid-intestine and ileum, and in order to study the various alterations occurring in the parasite prior to encystment, the following method was adopted: A large drop of fluid was taken from an emulsion made up of the contents of the colon and placed on a glass slide; a cover-glass was then placed on this drop, and the margin of the cover slip ringed round with melted paraffin so as to exclude the entrance of air; a suitable hanging-drop preparation was thus made, and no evaporation occurred. If such a preparation be examined, the flagellates will be seen to show active movements for two or three hours. Several things may happen; the parasite may undergo longitudinal division once more, or, what is more common, it becomes detached from the lining membrane of the gut and is a free-moving flagellate. After two or three hours its movements begin to get sluggish and finally its position on the slide becomes a fixed one with only rhythmical wave-like contractions passing along from the posterior end to the flagellum. During these

Cyst formation

[1] Minchin, E. A. (1908), "Investigations on the development of Trypanosomes in the Tsetse fly and other Diptera." *Quarterly Journal Microscopic Science*, Vol. LII., pp. 159-260.

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Post-Paramastix Stages
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[1] Minchin, E. A. (1909). "Investigations on the Development of Trypanosomes in the Tsetse fly and other Diptera." *Quarterly Journal Microscopical Science*, Vol. LIV., pp. 159–260.

contractions refractile masses can be seen passing along the flagellum for a distance of 5 μ or 6 μ. As these movements become more sluggish, granules are seen collecting in the posterior portion of the parasite. The anterior portion then commences to enlarge and dilate and becomes quite globular in shape; this enlargement and dilatation extend back to the posterior portion of the parasite and a complete alteration in its whole contour is thus produced (Plate XI., fig. 16); what was previously a cigar-shaped body has now become an irregular shaped cell with the flagellum still attached. That portion of the cell from which the flagellum arises retains for a short time its original conical shape.

Division of the kineto- and trophonuclei

Division of the kineto- and trophonuclei then occurs, the respective divided portions going to opposite sides of the cell, and finally the latter splits by a line of cleavage passing from the anterior to the posterior end. The two cells lie adjacent to each other, the original one still retaining the flagellum. Before the original cell has divided a large number of vacuoles and granules appear, the latter showing very marked Brownian movement.

During the whole of this stage, which occupied about an hour, the flagellum remained practically stationary, and longitudinal splitting of it was never observed. At regular intervals the flagellum was measured. Its intra-cellular portion showed a distinct curve, and this was associated with a diminution in length of the extra-cellular portion. This diminution continued to go on till the flagellum had shortened by 4–5 μ—and one concluded that this shortening was due to withdrawal of the flagellum within the cell. After this preliminary withdrawal the flagellum underwent a change in appearance, became more hyaline and often showed at its terminal end a granule. Finally, it ruptured from the anterior end of the cell and was disintegrated. Further divisions of the cell occurred till oval or pyriform bodies, about 2 μ in length, were formed. These represented the cysts which were found in large numbers in the fæces of *Lygæus militaris*. These cysts resembled in appearance the cysts found in the crop and require no further description. This cystic stage concluded the life-cycle of the herpetomonas found in *Lygæus militaris*.

Method of Infection

The method of infection is brought about by the accidental ingestion of the cysts passed in the fæces; the latter are deposited on the surface of plants, and infection is thus brought about.

Infection of *Lygæus* not hereditary

The parasites were only found in the alimentary canal of *Lygæus militaris*. The ova of a large number of infected bugs were examined and in no case were herpetomonads found; so one concluded that infection is not transmitted by heredity. This was borne out by the simple experiment of removing the eggs, laid by an infected female bug, to a clean jar. In about a week the larvæ hatched out, and in the course of a month or so became adult bugs. These were dissected at various stages of their growth, and in no case did they show infection with herpetomonads.

Culture and Animal Experiments

Owing to the close similarity that exists between *Herpetomonas lygæi* and the Leishman body of kala-azar, it occurred to one that the relationship between the two was a closer one than their mere morphology suggested, and that the presence of the flagellates, found in the bug, might in some way account for a disease like kala-azar which exists in certain parts of the Sudan. Accordingly, a number of inoculations and cultural experiments was carried out.

Emulsions of the crop contents of a heavily infected bug were injected into the

No demonstrable connection with the parasite of Kala-azar or Oriental Sore

peritoneal cavity of a *Cercopithecus* monkey, a species of monkey which can be artificially inoculated with kala-azar. Examinations of the peripheral and splenic blood of this monkey were carried out at various intervals, but all with negative results, and as the monkey showed no clinical signs of kala-azar the result of this experiment may be considered a negative one. A second experiment was carried out in which the fæces, containing a heavy infection of herpetomonas cysts, were injected into the peritoneal cavity of another monkey. This experiment, like the previous one, yielded a negative result. Experiments on the same lines were carried out on rabbits and on one guinea-pig, and they also gave negative results.

Cultivation experiments were carried out on different culture media, the best results being obtained by using Nicolle's medium at a temperature of 25° C. In this medium, the flagellates lived and multiplied for a period of three days, but subsequently lost their motility and became disintegrated. A temperature of 37° C. did not appear a suitable one for their growth.

Quite recently, oriental sore has been found to exist in the Sudan, and, with the view of ascertaining whether any relationship existed between the parasites of that disease and those found in the gut of *Lygæus militaris*, the following experiment was carried out. The fæces and crop contents of a heavily infected *Lygæus militaris* were placed on the surface of an abrasion artificially made on the leg of a *Cercopithecus* monkey. Living flagellates were found to be present at the end of six hours, after which they became disintegrated, and, at the end of 24 hours, no trace of either flagellates or cysts were visible in the lesion. A further examination of this lesion, carried out a week later, was also attended with negative results.

Patton, in his researches on kala-azar in India, found that the Leishman body underwent development changes in *Cimex rotundatus*, and naturally concluded that this bug played a possible rôle as a transmitting agent of the disease. In the Sudan, *Cimex rotundatus*, until recently, had only been found on the Red Sea coast,* but the allied species, *Cimex lectularius*, is practically ubiquitous, and in order to determine whether the latter bug was a host for flagellates of the herpetomonas type a large number of them was dissected, but all with negative results. A number of these living bugs and their nymphs was placed in a jar containing *Lygæi militaris*, the fæces of which were full of flagellates. After three or four days an interesting fact was noticed; some of the bugs were found attached to the bodies of the *Lygæi militaris* and appeared to be sucking their contents; the bugs were dissected, but in no case were flagellates or cysts discovered in their alimentary tracts. Mention may be made here that several feeding and transmission experiments, based on Patton's[1] line of work in India, have been carried out with *Cimex lectularius* and *Ornithodoros savignyi* on cases of kala-azar in the Sudan, and have all given negative results, and one is inclined to consider that these insects cannot be held responsible agents in the transmission of kala-azar in the Sudan.

Conclusion

In conclusion, one may say that the herpetomonas which is found to infest the alimentary tract of *Lygæus militaris* in the Sudan is more or less morphologically identical with, and goes through the same life-cycle as, the *Herpetomonas lygæi* described by Patton in India. Animal experiments proved it to be non-pathogenic, and, therefore, it is not at all likely that it is in any way associated with the pathological conditions known as oriental sore and kala-azar.

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* Bed-bugs recently sent me from the Lado District, Mongalla Province, proved to belong to this species.—A.B.

[1] Patton, W. S. (1907), "The Development of the Leishman-Donovan parasite in *Cimex rotundatus*." *Scientific Memoirs of the Govt. of India*, No. 31.

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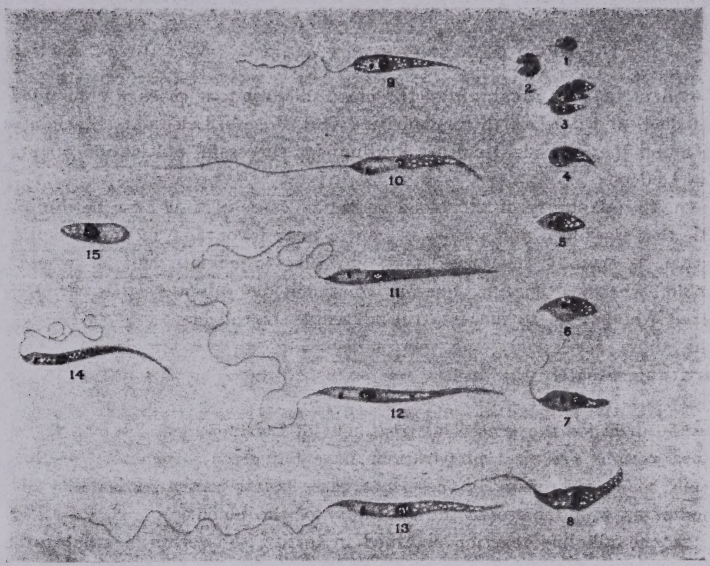
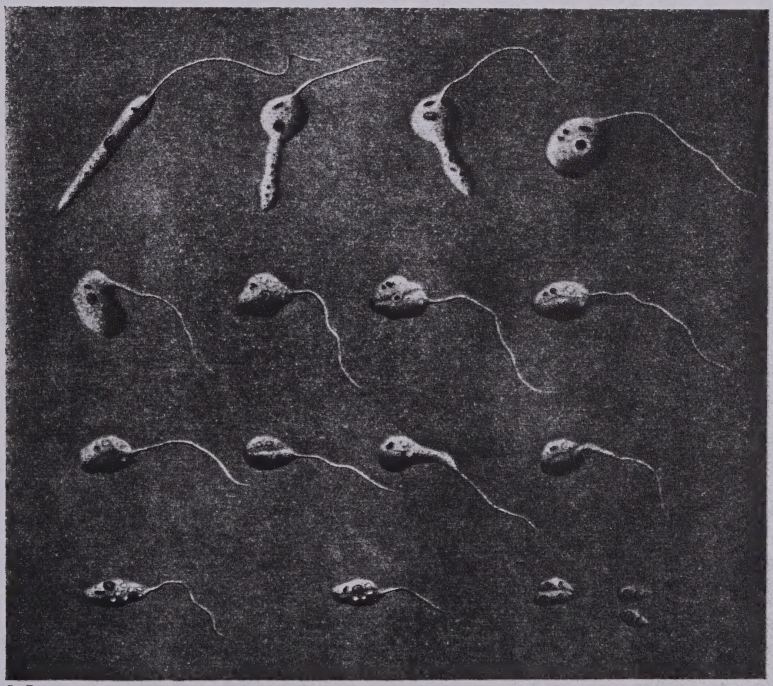


G. Buchanan

FLAGELLATE FORM OF *Herpetomonas lygæi*

1–6. Bodies resembling the Leishman-Donovan parasite. 7–9. Intermediate stages.
10–14. Fully-formed stages. 15. Form without visible kinetonucleus.
× 750 *diam.*



G. Buchanan

16. DIAGRAMMATIC ILLUSTRATION OF CYST FORMATION OF *Herpetomonas lygæi* IN THE HIND GUT OF *Lygæus militaris*, AS SEEN IN A HANGING-DROP PREPARATION
× *approx.* 1000 *diam.*